Screening Day-Old Blood Specimens for Hyperglycemia

LEONARD A. DOBRICK, B.S.

WITH the improved control of communicable diseases in the United States, medical and health organizations have increasingly focused their attention on the control and curtailment of chronic malfunctions. Among these is diabetes mellitus.

Early diagnosis and management of diabetes improve prognosis, and therefore, means for its early discovery are highly desirable. Through various promotional and educational measures, people are encouraged to submit voluntarily to blood sugar testing in mass screening programs designed to detect hyperglycemic individuals within a population. In these programs, on the thesis that individuals with elevated blood sugar are prone to diabetes, persons whose blood sugar exceeds a prescribed level are referred to their physicians for study and diagnosis. "Positive" cases so disclosed have yielded a number of previously unknown diabetics. Diabetes screening, consequently, has gained steadily in favor as a valuable adjunct to public health programs.

The effectiveness of this kind of mass screening has unfortunately been limited by lack of public participation. A steady flow of screenees, preferably large in number, is desirable if not essential for the success of such a program. Therefore, a means of screening which would be independent of the public's whim to participate would have its advantages.

Blood specimens received by public health laboratories for serologic testing for syphilis offer a readily available source of material for testing for hyperglycemia. In Milwaukee a substantial number of these specimens are approximately 24 hours old when tested in the health department laboratories.

Procedures for concurrent screening for both sugar and syphilis have relied on procuring dual specimens or have involved special serologic techniques because of added preservatives (*1,2*). Since diagnostic laboratories as a rule have little control over the manner in which specimens are drawn, dual sampling is often impracticable, and special techniques may prove unacceptable over established methods.

This paper describes studies carried out in the Milwaukee Health Department on the possibility of using day-old serologic blood specimens, as ordinarily received by the laboratories, for mass sugar testing. Because such specimens are submitted without a preservative, glycolysis would preclude screening at conventional levels of 130 mg./100 ml. and above. The investigation purports to demonstrate that, by lowering the screening level, day-old blood specimens, despite glycolysis, can be utilized to help differentiate normal from hyperglycemic persons.

Materials and Methods

For convenience, blood specimens were obtained solely from the health department's medical clinic, which routinely examines prospective civil service employees, food handlers, bartenders, persons requesting eugenic examinations, and others. Only preemployment candidates, however, were selected for this in-

Mr. Dobrick is a chemist in the bureau of laboratories, Milwaukee Health Department, Milwaukee, Wis.

vestigation because they were more readily available for followup.

In order to duplicate as nearly as possible the manner in which blood specimens are ordinarily procured and handled, specimens received no special treatment, and no special techniques were employed in processing them. Five to eight milliliters of venous blood, drawn at random hours after screenees had eaten, were transferred to a glass vial containing neither preservative nor anticoagulant. At the end of the day the specimens were placed in a refrigerator. On the following morning syphilis serology was performed on a portion of each specimen. The remainder was screened at a predetermined glucose level by an enzymatic method developed by the author (*3*). Screening, consequently, was carried out 22 to 30 hours after blood specimens were drawn. For periods of one-third to one-half of this time they were kept at room temperature, which varied seasonally from 70° to 90° F. During the remaining time they were kept in the refrigerator at 40° F.

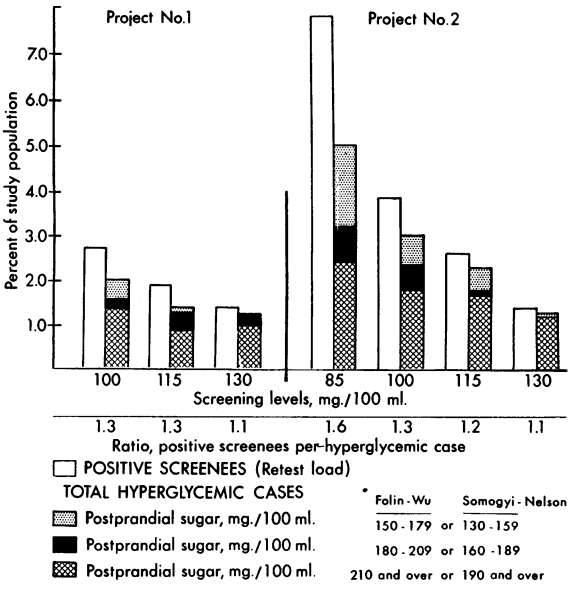
Because of the lapse of time between drawing and screening specimens, glycolysis had to be considered in determining the screening level to be used. An earlier study by our laboratories had indicated that, whereas the blood sugar of hyperglycemic individuals still exceeded 100 mg./100 ml. after 24 hours, that of normal individuals almost invariably dropped below this figure. Therefore, the screening level for the present study was set initially at 100 mg./100 ml. However, results later indicated that a lower level might prove more effective in finding hyperglycemic cases. Consequently, the first study (project 1) of this report covers a period of 7 months, September 1958–March 1959, during which 1,004 specimens were screened at 100 mg./100 ml. The second (project 2) extends over a period of 5 months, April–August 1959, during which an additional 1,002 specimens were screened at 85 mg./100 ml.

Table 1. Percentage distribution of population screened for hyperglycemia, according to age and sex, Milwaukee, Wis., September 1958–August 1959

Age (years)	Project 1 [1]			Project 2 [2]		
	Male	Female	Total	Male	Female	Total
20 and under	1.6	4.5	6.1	2.1	3.8	5.9
21–30	34.8	23.3	58.1	38.3	14.8	53.1
31–40	6.4	12.7	19.1	15.9	5.2	21.1
41–50	2.6	9.8	12.4	10.9	4.1	15.0
Over 50	1.8	2.5	4.3	1.9	3.0	4.9
Total	47.2	52.8	100.0	69.1	30.9	100.0

[1] 1,004 screenees; screening level 100 mg./100 ml.
[2] 1,002 screenees; screening level 85 mg./100 ml.

Percentage of screenees positive at various screening levels, and yield and distribution of hyperglycemic cases, Milwaukee Health Department, Milwaukee, Wis.



The age of screenees ranged from 17 to 65 years. More than half the population of both projects were less than 31 years old (table 1). However, 5 percent more screenees in project 2 than in project 1 were above 30 years of age and 3 percent more were above 40 years. Whereas male and female subjects were quite evenly divided in the first project, more than twice as many men as women were screened in the second.

Blood specimens which exceeded prescribed screening levels were designated as positive. The amount of glucose in positive specimens was established quantitatively by the Somogyi-Nelson method whenever sufficient serum was available.

To determine the relationship between positive screenees and true hyperglycemics, individuals screening positive were recalled and given a modified glucose tolerance test, usually on both a fasting and a postprandial blood specimen. The postprandial sample was taken 1 hour after oral administration of 100 gm. of glucose. Glucose feeding was omitted, however, when fasting blood sugar exceeded 200 mg./100 ml. The blood sugar level of each person retested was determined immediately by both the Folin-Wu and Somogyi-Nelson methods.

Whereas it has been established that postprandial blood is more reliable than fasting blood for detecting hyperglycemic individuals (*4,5*), our results are evaluated on the basis of the 1-hour postprandial blood sugar test. Retested persons whose blood sugar exceeded 150 mg./100 ml. by the Folin-Wu or 130 mg./100 ml. by the Somogyi-Nelson method were regarded as hyperglycemic. Such criteria represent a composite of values which have been generally recognized as indicative of hyperglycemia (*5–9*). No attempt was made to diagnose clinically any hyperglycemic cases found.

Results

In the first project the 27 persons, 2.7 percent, who failed the screening test constituted the retest load. In the second project the retest load was nearly tripled; 78, or 7.8 percent, were positive. Eighty percent of the positive screenees in project 1 and 85 percent of those in project 2 returned for glucose tolerance tests. Eighteen persons in project 1 and 42 in project 2 had above-normal postprandial blood sugar. Extrapolation of these data to full retest loads indicated that if all positive screenees had been retested, 20 hyperglycemic cases would have been the likely yield in the first project and 50 in the second. The ratio of positive screenees to hyperglycemic cases was 1.3 to 1 for project 1 and 1.6 to 1 for project 2. The yield of hyperglycemic cases, their apportionment among three blood sugar groups, and the retest loads are shown in table 2.

Depending upon the degree of blood sugar elevation, hyperglycemic cases were divided into the following groups:

Group	*Folin-Wu method (mg./100 ml.)*	*Somogyi-Nelson method (mg./100 ml.)*
A	150–179	130–159
B	180–209	160–189
C	210 and over	190 and over

Statistics compiled by the Public Health Service (*4*) from data on conventional screening indicate the mean specificity ratings of these groups as 95.0, 99.7, and 100 percent, respectively. In other words, whereas approximately 5.0 percent of true nondiabetics may still turn up in group A, only 0.3 percent are

Table 2. Classification of positive screenees, according to 1-hour postprandial blood sugar level, Milwaukee Health Department, Milwaukee, Wis.

Classification	Postprandial blood sugar [1]		Percent of study population			
			Project 1 [2] (N= 1,004)		Project 2 [3] (N= 1,002)	
	Folin-Wu method	Somogyi-Nelson method	Found	Extrapolated [4]	Found	Extrapolated [4]
Total retested			2. 4	2. 7	6. 6	7. 8
Normal	<150	<130	0. 6	0. 7	2. 4	2. 8
Hyperglycemic	>150	>130	1. 8	2. 0	4. 2	5. 0
Group A	150–179	130–159	. 4	. 5	1. 5	1. 8
Group B	180–209	160–189	. 2	. 2	. 7	. 8
Group C	210 and over	190 and over	1. 2	1. 3	2. 0	2. 4

[1] Milligrams per 100 ml. of blood.
[2] Screening level, 100 mg./100 ml.
[3] Screening level, 85 mg./100 ml.
[4] To total retest load.

likely to appear in group B, and none in group C.

In the present study 78 percent of the hyperglycemic cases found by screening at the 100 mg./100 ml. level fell into the high specificity groups B and C, compared with 64 percent at the 85 mg./100 ml. level. Whereas 10 out of 13 positive screenees in the first project had above-normal postprandial sugar, only 10 out of 16 in the second project fell within this category. Although a more favorable relationship between positive screenees and hyperglycemic cases was found at the higher screening level, the overall yield of hyperglycemics was far greater at the lower level. Consequently, while chances of retesting true normals were reduced at the higher screening level, the chances of missing hyperglycemic cases were greatly increased.

Inasmuch as the blood sugar of nearly all persons screening positive was confirmed quantitatively, screening performance could be evaluated at levels higher than those described. Results of screening at levels ranging from 85 to 130 mg. per 100 ml. are presented in the chart, which permits comparisons within, as well as between, the two populations studied. For comparison the yields of hyperglycemic cases have been extrapolated to full retest loads. The chart clearly illustrates that, as the screening level is elevated, the greater the certainty that the retest load will consist entirely of hyperglycemic cases, but only at considerable sacrifice to the yields obtained. Thus, when the screening level was raised from 100 to 130 mg. per 100 ml., losses were 37 percent in the first project and 56 percent in the second. The most pronounced loss, however, occurred between the 85 and 100 mg. levels in project 2. Here alone the yield of hyperglycemic cases was reduced by 40 percent.

Discussion

The Milwaukee Health Department receives approximately 50,000 blood specimens annually for syphilis serology. About one-half of these specimens are 1 day old when examined. The remainder are 48 to 72 hours old and, therefore, have not been considered in this study. It is estimated that in a community such as Milwaukee between 20,000 and 25,000 individuals could be screened annually for hyperglycemia by the method proposed in this report.

The specimens ordinarily received for syphilis serology probably would represent a fairer cross section of the adult population of the city than the specimens from the prospective civil service employees screened in this investigation, which represent only about one-tenth of the day-old specimens examined in the health department laboratory.

Even though confined solely to preemployment candidates, the populations of the study projects differed in age and sex distribution. Despite these differences, at any given screening level, the ratio of positive screenees to hyperglycemic cases was practically identical in both projects (chart). This suggests that under routine screening this factor would be independent of variances in the populations. Therefore, the ratios found in any screening program probably would be substantially the same as those determined in this study. It is also significant that the relationship between positive screenees and hyperglycemic cases at any given screening level remained unchanged despite the seasonal temperature differences under which the projects were executed. Contrary to what might have been expected, in view of glycolysis, the number of positive screenees as well as the yield of hyperglycemic cases was actually greater during the warm spring and summer months, when project 2 was carried out, than during the cooler fall and winter months of project 1. Seasonal temperature variations to which blood specimens may be subjected apparently would not preclude year-round screening.

Although the actual number of cases of diabetes among the screenees remains undetermined, results show that a significant number of persons with postprandial hyperglycemia can be detected by screening ordinary day-old serologic blood specimens. There can be little doubt that examination of these specimens can pinpoint individuals who warrant further study by their physicians. Results of this study demonstrate that such cases can be ascertained without excessively high retest loads.

For screening day-old blood with the greatest assurance that positive screenees are likely

to be diabetic, screening levels between 100 and 130 mg. per 100 ml. are suggested. If, however, one is chiefly concerned with ascertaining the greatest number of suspected diabetics, including those with blood sugar only slightly above normal, screening levels between 85 and 100 mg. per 100 ml. are recommended. Wilkerson (*6,10*) points out that future diabetics are to be found in the borderline group. His findings disclose that "persons with . . . blood sugar levels 'a little higher than normal' develop diabetes eight times oftener than those with normal blood sugar levels . . ."

The screening of day-old serologic blood specimens affords the public health officer another approach to detection of hyperglycemia on a broad basis. Because they are readily available in relatively large numbers, such specimens lend themselves well to this type of program.

Summary

The use of ordinary day-old serologic specimens of blood for mass screening has been proposed as a means of detecting hyperglycemia. In Milwaukee, Wis., serologic blood specimens from two study populations, each numbering approximately 1,000, were tested at levels ranging from 85 to 130 mg. per 100 ml. Results demonstrated that by lowering the screening levels below those conventionally employed, such specimens can be utilized to differentiate the normal from the hyperglycemic individual. The use of these specimens in a diabetes screening program appears to be both practical and feasible.

REFERENCES

(*1*) Pendleton, J. L.: Piggyback blood testing. Pub. Health Rep. 71: 622, June 1956.

(*2*) Jolly, J. J., White, W. V., Portnoy, J., and Gutridge, J. W.: Blood sugar and syphilis serology using a single specimen. Pub. Health Rep. 75: 115–118, February 1960.

(*3*) Dobrick, L. A.: Screening method for glucose of blood serum utilizing glucose oxidase and an indophenol indicator. J. Biol. Chem. 231: 403–409, March 1958.

(*4*) U.S. Public Health Service: Diabetes program guide. PHS Publication No. 506. U.S. Government Printing Office, Washington, D.C., revised, 1960.

(*5*) Wilkerson, H. L. C., and Krall, L. P.: Diabetes in a New England town. A study of 3,516 persons in Oxford, Mass. J.A.M.A. 135: 209–216, Sept. 27, 1947.

(*6*) Wilkerson, H. L. C., and Krall, L. P.: Diabetes in a New England town. Report of four-year progress study of the Oxford, Mass., diabetes survey of 1946–1947. J.A.M.A. 152: 1322–1329, Aug. 1, 1953.

(*7*) Conn, J. W.: The prediabetic state in man. Diabetes 7: 347–357, September–October 1958.

(*8*) Mosenthal, H. O., and Barry, E.: Criteria for and interpretation of normal glucose tolerance tests. Ann. Int. Med. 33: 1175–1193, November 1950.

(*9*) Macy, D., Frank, M. E., and Baumann, F.: Evaluation of random blood glucose levels among ambulant women. J.A.M.A. 168: 2010–2012, Dec. 13, 1958.

(*10*) Wilkerson, H. L. C., Krall, L. P., and Butler, F. K.: Diabetes in a New England town. III. A comprehensive baseline study in Oxford, Mass. J.A.M.A. 169: 910–914, Feb. 28, 1959.

Poison Prevention Week

The third week of March each year, beginning March 18, 1962, will be Poison Prevention Week, by act of Congress, approved by President Kennedy.

Materials for supporting the program, with sponsors in industry, government, and the health professions, may be obtained from Henry L. Verhulst, secretary, Steering Committee for National Poison Prevention Week, National Clearinghouse for Poison Control Centers, Public Health Service, U.S. Department of Health, Education, and Welfare, Washington 25, D.C.